

**What Is Claimed:**

1. A fibronectin type III (Fn3) polypeptide monobody comprising:  
at least two Fn3  $\beta$ -strand domain sequences with a loop region  
5 sequence linked between adjacent  $\beta$ -strand domain sequences; and  
optionally, an N-terminal tail of at least about 2 amino acids, a C-terminal tail of at least about 2 amino acids, or both;  
wherein at least one loop region sequence, the N-terminal tail, or the C-terminal tail comprises an amino acid sequence which varies by deletion,  
10 insertion, or replacement of at least two amino acids from a corresponding loop region, N-terminal tail, or C-terminal tail in a wild-type Fn3 domain of fibronectin, and  
wherein the polypeptide monobody exhibits nuclear receptor binding activity.
- 15 2. The polypeptide monobody according to claim 1, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.
- 20 3. The polypeptide monobody according to claim 2, wherein the nuclear receptor is a steroid receptor.
- 25 4. The polypeptide monobody according to claim 3, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progestin receptors, a glucocorticoid receptor, or a mineralocorticoid receptor.
- 30 5. The polypeptide monobody according to claim 4, wherein the steroid receptor is an estrogen receptor.
6. The polypeptide monobody according to claim 5, wherein the polypeptide monobody exhibits estrogen receptor binding activity in the presence of an estrogen receptor agonist or an estrogen receptor antagonist.

7. The polypeptide monobody according to claim 6, wherein the estrogen receptor agonist is estradiol, estriol, diethylstilbestrol, or genistein.

5 8. The polypeptide monobody according to claim 6, wherein the estrogen receptor antagonist is hydroxy tamoxifen, ICI182780, or raloxifene.

10 9. The polypeptide monobody according to claim 1, wherein said at least two Fn3  $\beta$ -strand domain sequences comprises  $\beta$ -strand domain sequences A through G of a wild-type tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop regions comprise an AB loop, a BC loop, a CD loop, a DE loop, and EF loop, and an FG loop.

15 10. The polypeptide monobody according to claim 9, wherein the at least one loop region sequence is selected from the group consisting of the AB loop region sequence, BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.

20 11. The polypeptide monobody according to claim 9, wherein the at least one loop region sequence is a combination of the BC loop region sequence and the FG loop region sequence.

25 12. The polypeptide monobody according to claim 1, wherein the wild-type Fn3 domain of fibronectin is a wild-type tenth Fn3 domain of human fibronectin.

30 13. A fusion protein comprising:  
a first portion comprising a polypeptide monobody according to  
claim 1 and  
a second portion fused to the first portion.

14. The fusion protein according to claim 13, wherein the second portion comprises a label.

15. The fusion protein according to claim 14, wherein the label is  
5 an alkaline phosphatase tag or a His<sub>(6)</sub> tag.

16. The fusion protein according to claim 13, wherein the second portion comprises a transcriptional activation domain.

10 17. A DNA molecule encoding the polypeptide monobody according to claim 1.

15 18. An expression vector comprising a DNA molecule according to claim 17.

19. A host cell comprising a heterologous DNA molecule according to claim 17.

20. The host cell according to claim 19, wherein the host cell is selected from the group consisting of a bacteria, a mammalian cell, and a yeast.

21. The host cell according to claim 20, wherein the host cell is a yeast.

25 22. A combinatorial library comprising:  
a plurality of fusion polypeptides each comprising a transcriptional activation domain fused to a distinct fibronectin type III (Fn3) polypeptide monobody, the polypeptide monobody comprising (i) at least two Fn3 β-strand domain sequences, (ii) a loop region sequence linked between adjacent β-strand domain sequences, and (iii) optionally, an N-terminal tail of at least about 2 amino acids, a C-terminal tail of at least about 2 amino acids, or both,  
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wherein at least one loop region sequence, the N-terminal tail, or the C-terminal tail comprises a combinatorial amino acid sequence which varies by deletion, insertion, or replacement of at least two amino acids from a corresponding loop region, N-terminal tail, or C-terminal tail in a wild-type Fn3 domain of fibronectin.

23. The combinatorial library according to claim 22, wherein the combinatorial amino acid sequence of each fusion polypeptide differs from the combinatorial amino acid sequence of substantially all other fusion polypeptides of said plurality thereof.

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24. The combinatorial library according to claim 22, wherein the at least two Fn3  $\beta$ -strand domain sequences comprises  $\beta$ -strand domain sequences A through G of a wild-type tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop regions comprise an AB loop, a BC loop, a CD loop, a DE loop, and EF loop, and an FG loop.

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25. The combinatorial library according to claim 24, wherein the at least one loop region sequence is selected from the group consisting of the AB loop region sequence, BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.

26. The combinatorial library according to claim 22, wherein the combinatorial amino acid sequence is at least about 5 amino acids in length.

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27. The combinatorial library according to claim 22, wherein the combinatorial amino acid sequence is at least about 10 amino acids in length.

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28. The combinatorial library according to claim 22, wherein the transcriptional activation domain is selected from the group consisting of B42 and Gal4 activation domains.

29. The combinatorial library according to claim 22, wherein the wild-type Fn3 domain of fibronectin is a wild-type tenth Fn3 domain of human fibronectin.

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30. An *in vivo* composition comprising:  
a fusion polypeptide of the combinatorial library according to  
claim 22;

10 comprising a target protein, or fragment thereof, fused to the C-terminus of a DNA-binding domain which binds to the 5' regulatory region of the reporter gene,  
wherein binding of the polypeptide monobody of the fusion polypeptide to the target protein, or fragment thereof, of the second fusion polypeptide brings the transcriptional activation domain of the fusion polypeptide in sufficient  
15 proximity to the DNA-binding domain of the second fusion polypeptide to induce expression of the reporter gene.

20 31. The *in vivo* composition according to claim 30, wherein the target protein, or fragment thereof, is a nuclear receptor or fragment thereof including a ligand-binding domain.

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32. The *in vivo* composition according to claim 31, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

33. The *in vivo* composition according to claim 32, wherein the nuclear receptor is a steroid receptor.

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34. The *in vivo* composition according to claim 33, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

35. The *in vivo* composition according to claim 34, wherein the steroid receptor is an estrogen receptor.

36. The *in vivo* composition according to claim 30, wherein the 5 reporter gene is a nutrient marker gene, a  $\beta$ -galactosidase gene, or a fluorescent protein gene.

37. The *in vivo* composition according to claim 30, wherein the at least two Fn3  $\beta$ -strand domain sequences comprises  $\beta$ -strand domain sequences A 10 through G of a wild-type tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop regions comprise an AB loop, a BC loop, a CD loop, a DE loop, and EF loop, and an FG loop.

38. The *in vivo* composition according to claim 37, wherein the at 15 least one loop region sequence is selected from the group consisting of the AB loop region sequence, BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.

39. The *in vivo* composition according to claim 30, wherein the 20 combinatorial amino acid sequence is at least about 5 amino acids in length.

40. The *in vivo* composition according to claim 30, wherein the combinatorial amino acid sequence is at least about 10 amino acids in length.

41. The *in vivo* composition according to claim 30, wherein the *in* 25 *vivo* composition is present in a bacteria, a mammalian cell, or a yeast cell.

42. The *in vivo* composition according to claim 30, wherein the 30 transcriptional activation domain is a B42 activation domain or a Gal4 activation domain.

43. The *in vivo* composition according to claim 30, wherein the DNA-binding domain is a LexA DNA-binding domain or a Gal4 DNA-binding domain.

5 44. A method of identifying a polypeptide monobody having target protein binding activity, said method comprising:

10 providing a host cell comprising (i) a reporter gene under control of a 5' regulatory region operable in the host cell, (ii) a first chimeric gene which encodes a first fusion polypeptide comprising a target protein, or fragment thereof, fused to a C-terminus of a DNA-binding domain which binds to the 5' regulatory region of the reporter gene, and (iii) a second chimeric gene which encodes a second fusion polypeptide comprising a polypeptide monobody fused to a transcriptional activation domain; and

15 detecting expression of the reporter gene, which indicates binding of the polypeptide monobody of the second fusion polypeptide to the target protein such that the transcriptional activation domain of the second fusion polypeptide is in sufficient proximity to the DNA-binding domain of the first fusion polypeptide to allow expression of the reporter gene.

20 45. The method according to claim 44, wherein the polypeptide monobody is a fibronectin type III (Fn3) polypeptide monobody comprising (i) at least two Fn3  $\beta$ -strand domain sequences with a loop region sequence linked between adjacent  $\beta$ -strand domain sequences, and (ii) optionally, an N-terminal tail of at least about 2 amino acids, a C-terminal tail of at least about 2 amino acids, or both; and  
25 wherein at least one loop region sequence, the N-terminal tail, or the C-terminal tail comprises an amino acid sequence which varies by deletion, insertion, or replacement of at least two amino acids from a corresponding loop region, N-terminal tail, or C-terminal tail in a wild-type Fn3 domain of fibronectin.

30 46. The method according to claim 45, wherein the fibronectin type III (Fn3) polypeptide monobody exhibits nuclear receptor binding activity and the

target protein is a nuclear receptor or fragment thereof including a ligand-binding domain.

47. The method according to claim 45, wherein the  
5 at least two Fn3  $\beta$ -strand domain sequences comprises  $\beta$ -strand domain sequences A through G of a wild-type tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop regions comprise an AB loop, a BC loop, a CD loop, a DE loop, and EF loop, and an FG loop.
- 10 48. The method according to claim 47, wherein the at least one loop region sequence is selected from the group consisting of the AB loop region sequence, the BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.
- 15 49. The method according to claim 44, wherein the transcriptional activation domain is selected from the group consisting of B42 and Gal4 activation domains.
50. The method according to claim 44, wherein the DNA-binding  
20 domain is a LexA DNA-binding domain or a Gal4 DNA-binding domain.
51. The method according to claim 44, wherein the host cell is a  
bacteria, a mammalian cell, or a yeast cell.
- 25 52. The method according to claim 44, wherein the reporter gene is a nutrient marker gene and said detecting comprises exposing host cells to a nutrient-deficient media and identifying host cell colonies that grow on the nutrient-deficient media.
- 30 53. The method according to claim 44, wherein the reporter gene is a  $\beta$ -galactosidase gene and said detecting comprises exposing host cells to X-gal and identifying host cell colonies exhibiting  $\beta$ -galactosidase activity.

54. The method according to claim 44, wherein the reporter gene is  
a fluorescent protein gene and said detecting comprises exposing the host cells to an  
excitatory light source and identifying host cells that emit light at a particular  
5 wavelength.

55. The method according to claim 44, wherein said providing  
comprises:

10 introducing into a host cell comprising a reporter gene, a first  
vector comprising the first chimeric gene and a second vector comprising the second  
chimeric gene.

15 56. The method according to claim 44, wherein the target protein,  
or fragment thereof, is a nuclear receptor or fragment thereof including a ligand-  
binding domain.

20 57. The method according to claim 44 further comprising:  
modifying the amino acid sequence of the polypeptide  
monobody identified during said detecting to produce a modified polypeptide  
monobody, and

repeating said providing and detecting, under more stringent  
conditions, with a modified second chimeric gene which encodes the modified  
polypeptide monobody.

25 58. A method of screening a candidate drug for nuclear receptor  
agonist or antagonist activity, said method comprising:

providing a host cell comprising (i) a reporter gene under  
control of a 5' regulatory region, (ii) a first chimeric gene which encodes a first fusion  
polypeptide comprising a nuclear receptor, or fragment thereof including a ligand-  
30 binding domain, fused to a C-terminus of a DNA-binding domain which binds to the  
5' regulatory region of the reporter gene, and (iii) a second chimeric gene which  
encodes a second fusion polypeptide comprising a polypeptide sequence fused to a

transcriptional activation domain, the polypeptide sequence binding to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist of the nuclear receptor, presence of an agonist of the nuclear receptor, presence of an antagonist of the nuclear receptor, or presence of both an agonist and an antagonist of the nuclear receptor;

5 growing the host cell in a growth medium comprising a candidate drug; and

detecting expression of the reporter gene, which indicates binding of the polypeptide sequence of the second fusion polypeptide to the nuclear receptor, or fragment thereof, such that the transcriptional activation domain of the second fusion polypeptide is in sufficient proximity to the DNA-binding domain of the first fusion polypeptide to allow expression of the reporter gene,

10 wherein modulation of reporter gene expression indicates that the candidate drug is either an agonist or an antagonist, or has mixed activity.

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59. The method according to claim 58, wherein an increase in reporter gene expression indicates that the candidate drug has agonist activity when the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of an agonist.

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60. The method according to claim 58, wherein an increase in reporter gene expression indicates that the candidate drug has antagonist activity when the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of the antagonist.

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61. The method according to claim 58, wherein said providing comprises providing four host cells and separately growing the four host cells on the same growth media containing the same candidate drug, and

30 wherein the first host cell comprises a second fusion polypeptide including a polypeptide sequence which binds the nuclear receptor in the presence of only a nuclear receptor agonist,

wherein the second host cell comprises a second fusion polypeptide including a polypeptide sequence which binds the nuclear receptor in the presence of only a nuclear receptor antagonist,

- wherein the third host cell comprises a second fusion
- 5    polypeptide including a polypeptide sequence which binds the nuclear receptor in the presence of both a nuclear receptor agonist and a nuclear receptor antagonist, and
- wherein the second host cell comprises a second fusion
- polypeptide including a polypeptide sequence which binds the nuclear receptor in the presence of neither a nuclear receptor agonist nor a nuclear receptor antagonist.

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62.    The method according to claim 58, wherein the polypeptide sequence is a polypeptide monobody.

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63.    The method according to claim 62, wherein the polypeptide monobody is derived from a tenth fibronectin type III domain of human fibronectin.

64.    The method according to claim 62, wherein the polypeptide sequence is present in a loop region sequence, N-terminal tail, or C-terminal tail of the polypeptide monobody.

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65.    The method according to claim 58, wherein the transcriptional activation domain is a B42 or Gal4 activation domains.

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66.    The method according to claim 58, wherein the DNA-binding domain is a LexA DNA-binding domain or a Gal4 DNA-binding domain.

67.    The method according to claim 58, wherein the reporter gene is a nutrient marker gene, a  $\beta$ -galactosidase gene, or a fluorescent protein gene.

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68.    The method according to claim 67, wherein the reporter gene is a nutrient marker gene and said detecting comprises exposing host cells to a nutrient-

deficient media and identifying host cell colonies that grow on the nutrient-deficient media.

69. The method according to claim 67, wherein the reporter gene is  
5 a β-galactosidase gene and said detecting comprises exposing host cells to X-gal and  
identifying host cell colonies exhibiting β-galactosidase activity.

70. The method according to claim 67, wherein the reporter gene is  
a fluorescent protein gene and said detecting comprises exposing the host cells to an  
10 excitatory light source and identifying host cells that emit light at a particular  
wavelength.

71. The method according to claim 58, wherein said providing  
comprises:  
15 introducing into a host cell comprising a reporter gene, a first  
vector comprising the first chimeric gene and a second vector comprising the second  
chimeric gene.

72. The method according to claim 58, wherein the nuclear receptor  
20 is selected from the group consisting of steroid receptors, thyroid receptors, retinoid  
receptors, vitamin D receptors, and orphan nuclear receptors.

73. The method according to claim 72, wherein the nuclear receptor  
is a steroid receptor.  
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74. The method according to claim 73, wherein the steroid receptor  
is an estrogen receptor, an androgen receptor, a progesterone receptors, a  
glucocorticoid receptor, or a mineralocorticoid receptor.

30 75. The method according to claim 58, wherein the host cell is a  
bacteria, a mammalian cell, or a yeast cell.

76. A kit comprising:

a culture system which includes a culture medium on which has been placed at least one transformed host cell, each of the at least one transformed host cell comprising (i) a reporter gene under control of a 5' regulatory region, (ii) a first chimeric gene which encodes a first fusion polypeptide comprising a nuclear receptor, or fragment thereof including a ligand-binding domain, fused to a C-terminus of a DNA-binding domain which binds to the 5' regulatory region of the reporter gene, and (iii) a second chimeric gene which encodes a second fusion polypeptide comprising a polypeptide sequence fused to a transcriptional activation domain, the polypeptide sequence binding to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist of the nuclear receptor, presence of an agonist of the nuclear receptor, presence of an antagonist of the nuclear receptor, or presence of both an agonist and an antagonist of the nuclear receptor.

15 77. The kit according to claim 76, wherein the at least one type of transformed host cell comprises:

a first transformed host cell comprising a second chimeric gene which encodes a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of a nuclear receptor agonist and

20 a second transformed host cell comprising a second chimeric gene which encodes a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of a nuclear receptor antagonist.

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78. The kit according to claim 77, wherein the first and second transformed host cells are strategically placed on the growth medium such that the first and second transformed host cells are physically separated from one another.

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79. The kit according to claim 77, wherein the at least one transformed host cell further comprises:

a third transformed host cell comprising a second chimeric gene which encodes a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist; and

5                   a fourth transformed host cell comprising a second chimeric gene which encodes a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of both an agonist and an antagonist.

10                 80.       The kit according to claim 79, wherein the third and fourth transformed host cells are strategically placed on the growth medium such that the third and fourth transformed host cells are physically separated from one another as well as the first and second transformed host cells.

15                 81.       The kit according to claim 76, wherein the polypeptide sequence is a polypeptide monobody.

82.       The kit according to claim 81, wherein the polypeptide monobody is derived from a tenth fibronectin type III domain of human fibronectin.

20                 83.       The kit according to claim 76, wherein the host cell is a bacteria, a mammalian cell, or a yeast cell.

25                 84.       A kit comprising:  
                      a plurality of host cells, each comprising a reporter gene under control of a 5' regulatory region and a heterologous DNA molecule encoding a first fusion polypeptide comprising a nuclear receptor, or fragment thereof which includes a ligand-binding domain, fused to a C-terminus of a DNA-binding domain which binds to the 5' regulatory region of the reporter gene; and

30                 a vector comprising a DNA molecule encoding a second fusion polypeptide comprising a transcriptional activation domain fused to a polypeptide monobody;

wherein, upon mutation of the DNA molecule to encode a mutant polypeptide antibody and

5 wherein upon introduction of the vector into at least a portion of said plurality of host cells, expression of the reporter gene is induced upon binding of the polypeptide monobody of the second fusion polypeptide to the nuclear receptor, or fragment thereof, of the first fusion polypeptide such that the transcriptional activation domain of the second fusion polypeptide is in sufficient proximity to the DNA-binding domain of the first fusion polypeptide.

10 85. The kit according to claim 84, wherein the polypeptide monobody is derived from the tenth fibronectin type III domain of human fibronectin.

86. The kit according to claim 84, wherein the host cell is a bacteria, a mammalian cell, or a yeast cell.

15 87. A method of validating target protein activity comprising:  
exposing a target protein to a polypeptide monobody which binds to the target protein and  
determining whether binding of the target protein by the  
20 polypeptide monobody modifies target protein activity.

88. The method according to claim 87, wherein said exposing is carried out *in vivo*.

25 89. The method according to claim 88, wherein said exposing is carried out in a yeast cell, bacterial cell, or mammalian cell.

90. The method according to claim 87, wherein said determining comprises:

30 detecting mRNA or protein expression levels prior to said exposing and after said exposing and

comparing the detected mRNA or protein expression levels to identify proteins which are downstream of the pathway in which target protein, wherein modified expression levels indicated modified target protein activity.

5               91.     The method according to claim 87, wherein the target protein is required for cell growth or survival, said determining comprising:

measuring cell growth or survival after said exposing, wherein reduced cell growth or survival indicates inhibition of target protein activity.

10              92.     The method according to claim 87, wherein the target protein is a pathogen protein involved in host-pathogen interaction, said exposing comprising:  
                  exposing a host cell comprising the polypeptide monobody to the pathogen.

15              93.     The method according to claim 87, wherein said determining comprises:  
                  determining the extent of pathogen-induced disease progression in the host cell.

20              94.     The method according to claim 93, wherein the pathogen is a virus or a bacteria.

95.     The method according to claim 87, wherein said exposing comprises:

25              co-expressing in a single cell comprising a reporter gene under control of a 5' regulatory region, (i) a first fusion polypeptide comprising a transcriptional activation domain fused to a target protein co-activator which binds the target protein, (ii) a second fusion polypeptide comprising a target protein fused to a C-terminus of a DNA-binding domain which binds to the 5' regulatory region of the reporter gene, and (iii) a polypeptide monobody which binds the target protein,

wherein the absence of reporter gene expression indicates that the polypeptide monobody effectively inhibits the activity of the target protein and the target protein co-activator.

5           96.     A method of measuring polypeptide monobody binding affinity for a target protein, said method comprising:

              exposing a target protein to an interaction partner which binds the target protein and a polypeptide monobody which binds the target protein and  
              measuring the degree to which the polypeptide monobody

10        competes with the interaction partner.

97.     The method according to claim 96, wherein said exposing is carried out *in vitro*.

15        98.     The method according to claim 97, wherein the target protein is bound to a substrate.

99.     The method according to claim 97, wherein the polypeptide monobody comprises a label.

20        100.    The method according to claim 99, wherein the label is an alkaline phosphatase tag or a His<sub>(6)</sub> tag.

101.    The method according to claim 96, wherein said exposing is  
25        carried out *in vivo*.

102.    The method according to claim 101, wherein said exposing comprises:

              co-expressing in a cell comprising a reporter gene under control  
30        of a 5' regulatory region, (i) a first fusion polypeptide comprising a transcriptional activation domain fused to a target protein co-activator which binds the target protein,  
              (ii) a second fusion polypeptide comprising the target protein fused to a C-terminus of

a DNA-binding domain which binds to the 5' regulatory region of the reporter gene, and (iii) a polypeptide monobody which binds the target protein,

wherein reduced reporter gene expression as compared to a cell which does not contain the polypeptide monobody indicates that the polypeptide monobody effectively competes with the interaction partner for binding to the target protein.

103. A method of modulating target protein activity comprising:  
exposing a target protein to a polypeptide monobody which  
10 binds the target protein under conditions effective to modify target protein activity.

104. The method according to claim 103, wherein said exposing is carried out *in vivo*.

15 105. The method according to claim 104, wherein said exposing is carried out in a yeast cell, bacterial cell, or mammalian cell.

106. The method according to claim 103, wherein the target protein is selected from the list of target proteins as disclosed herein.

20 107. The method according to claim 103, wherein the polypeptide monobody comprises a localization signal for retention of the target protein in the endoplasmic reticulum.

25 108. The method according to claim 107, wherein the localization signal comprises a KDEL amino acid sequence secured via peptide bond to the C-terminal end of the polypeptide monobody.